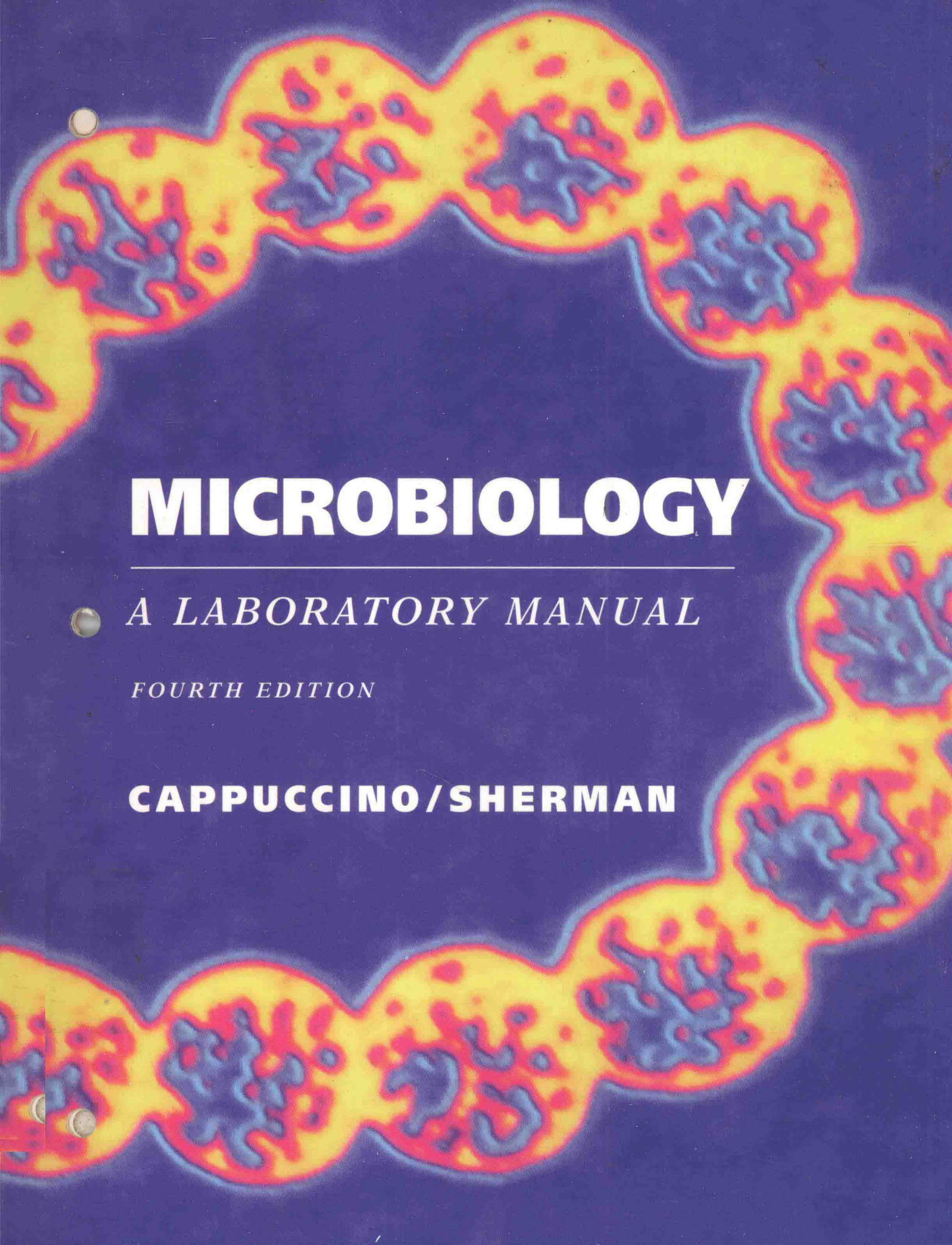
MICROBIOLOGY
A LABORATORY MANUAL
FOURTH EDITION
CAPPUCCINO/SHERMAN

MICROBIOLOGY

A Laboratory Manual

Fourth Edition

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The authors and publisher believe that the laboratory experiments described in this publication, when conducted in conformity with the safety precautions described herein and according to the school's laboratory safety procedures, are reasonably safe for the students for whom this manual is directed. Nonetheless, many of the described experiments are accompanied by some degree of risk, including human error, the failure or misuse of laboratory or electrical equipment, mismeasurement, spills of chemicals, and exposure to sharp objects, heat, bodily fluids, blood, or other biologics. The author and publisher disclaim any liability arising from such risks in connection with any of the experiments contained in this manual. If students have questions or problems with materials, procedures, or instructions on any experiment, they should always ask their instructor for help before proceeding.

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Preface

Microbiology is a dynamic science. It is constantly evolving as more information is added to the continuum of knowledge, and as microbiological techniques are rapidly being modified and refined. In this fourth edition of *Microbiology: A Laboratory Manual,* we have continued to provide a blend of traditional methodologies with more contemporary procedures to meet the pedagogical needs of all students studying microbiology. As in previous editions, this fourth edition contains a large number of diverse experimental procedures, providing instructors with the flexibility to design a course syllabus that meets their particular instructional approach. We have focused on the modification, amplification, and clarification of background information as well as the experimental procedures to facilitate student understanding and ease of performance of microbiological procedures. In addition, the color-photo insert is expanded to enhance student interpretation of their observed experimental data.

The structure of the manual remains essentially the same as in the earlier editions. Comprehensive introductory material is given at the beginning of each major area of study, and specific explanations and detailed directions precede each experiment. We feel that this approach will augment, enhance, and reinforce course lectures, thereby enabling students to more readily comprehend the concepts and purposes of each experiment. This will be a further asset to those in institutions in which the laboratory and lecture sections are not taught concurrently. Finally, we feel that this book should reduce the time required for explanations at the beginning of each laboratory session and thus make more time available for performing the experiments.

The wide variety of experiments were critically selected and tested to facilitate effective instruction in the basic principles and techniques in a variety of microbiological areas. Thus, this laboratory manual provides a wide spectrum of exercises suitable for use in elementary and advanced general microbiology courses as well as in allied health programs. Also, we have carefully designed procedures so that the supplies, equipment, and instrumentation commonly found in undergraduate institutions will suffice for their successful execution.

The manual consists of 77 exercises arranged in 15 parts. The exercises have been reorganized in this edition to progress from those that are basic and introductory, requiring minimal manipulations, to those that are more complex, requiring more sophisticated skills.

Part I, on **basic laboratory techniques for isolation, cultivation, and cultural characterization of microorganisms,** introduces basic procedures used for isolation and cultivation of microorganisms.

Part II, on **microscopy,** introduces the use and care of the microscope for the study of microorganisms.

Part III, on **bacterial staining,** focuses on procedures for visualization and differentiation of microorganisms and cell structures.

Part IV focuses on **cultivation of microorganisms, nutritional and physical requirements, and enumeration of microbial populations.**

Part V, on **biochemical activities,** introduces the varied cellular enzymatic activities that may be used for differentiation and identification of specific groups of microorganisms.

Parts VI, VII, and VIII introduce the areas of **protozoology, mycology,** and **virology.**

Part IX, **control of microbial growth,** discusses the antimicrobial activities of various physical and chemical agents.

Parts X and XI are concerned with the sanitary aspects of **water** and **food** as well as the fermentative role of microorganisms in the production of some beverages and food products.

Part XII, on the **microbiology of soil,** discusses the role of soil microorganisms in the nitrogen cycle and antibiotic production.

Part XIII, on **bacterial genetics,** presents selected experiments to illustrate genetic principles using bacterial systems.

Parts XIV and XV, on **medical microbiology** and **immunology,** highlight both the conventional and the more recent rapid clinical screening methodologies used for the isolation and identification of pathogenic microorganisms.

The format of each exercise is intended to facilitate presentation of the material by the instructor and to maximize the learning experience. To this end, each experiment is designed as follows:

Purpose: Defines the specific principles and/or techniques to be mastered.

Principle: An in-depth discussion of the microbiological concept or technique and the specific experimental procedure.

Materials: To facilitate the preparation of all laboratory sessions, a list of the following materials appears under this heading:

Cultures: These are the selected test organisms that have been chosen to demonstrate effectively the experimental principle or technique under study, as well as their ease of cultivation and maintenance in stock culture. A complete listing of the experimental cultures and prepared slides is presented in Appendix 4.

Media: These are the specific media and their quantities per designated student group. Appendix 1 lists the composition and method of preparation of all the media used in this manual.

Reagents: These include biological stains as well as test reagents. The chemical composition and preparation of the reagents is presented in Appendices 2 and 3.

Equipment: Listed under this heading are the supplies and instrumentation that are needed during the laboratory session. The suggested equipment was selected to minimize expense.

Procedure: Explicit instructions augmented by diagrams aid in the execution and interpretation of the experiments.

Observations and Results: Tear-out sheets located at the end of each exercise facilitate interpretation of data and subsequent review by the instructor.

Review Questions: Questions on tear-out report sheets aid the instructor in determining the student's ability to understand the experimental concepts and techniques. Questions that call for more critical thinking are indicated by the symbol shown to the left.

Finally, we hope that this manual will serve as a vehicle for the development of manipulative skills and techniques essential for understanding the integrated complexity of the biochemical structure and function of the single cell. This will enable an extension of these principles toward a better understanding of the more complex, higher

forms of life. Ultimately, we hope that some students might further pursue the study of life at the molecular level or apply these laboratory skills in the vocational fields of applied microbiology and allied health.

James G. Cappuccino
Natalie Sherman

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We wish to express our sincere gratitude to the following individuals for their constructive criticisms and invaluable suggestions during the preparation of this fourth edition: Susan Morrison, College of Charleston; Robert D. Simon, SUNY at Geneseo; Candy Coffin, Briar Cliff College, Iowa; and Liz Gerity, Elgin Community College, Illinois.

Likewise, we wish to extend our appreciation to the staff at The Benjamin/Cummings Publishing Company, whose expertise and technical skills have guided us over the many years. Special thanks are extended to the following individuals, who have so expertly guided us in the preparation of this edition: Leslie With for her editorial acumen; Marjorie Wexler for her meticulous copyediting of the manuscript; and Larry Olsen and Brian Jones for their efficient management of the production process.

Last, but certainly not least, we wish to express our gratitude to the microbiology laboratory technicians at Rockland Community College—Ms. Joan Grace, who early on assisted us in performing all the experiments to ensure their success when performed by the students, and Ms. Roz Wehrman, who is presently following in Joan's footsteps.

Laboratory Safety: General Rules and Regulations

A rewarding laboratory experience demands strict adherence to prescribed rules for personal and environmental safety. The former reflects concern for your personal safety in terms of avoiding laboratory accidents. The latter requires that you maintain a scrupulously clean laboratory setting to prevent contamination of experimental procedures by microorganisms from exogenous sources.

Because most microbiological laboratory procedures require the use of living organisms, an integral part of all laboratory sessions is the use of aseptic techniques. Although the virulence of microorganisms used in the academic laboratory environment has been greatly diminished because of their long-term maintenance on artificial media, **all microorganisms should be treated as potential pathogens** (organisms capable of producing disease). Thus, microbiology students must develop aseptic techniques (free of pathogenic organisms) in preparation for industrial and clinical marketplaces where manipulation of infectious organisms may be the norm rather than the exception.

The following basic steps should be observed at all times to reduce the ever-present microbial flora of the laboratory environment.

1. Upon entering the laboratory, place coats, books, and other paraphernalia in specified locations—never on bench tops.
2. Keep doors and windows closed during the laboratory session to prevent contamination from air currents.
3. At the beginning and termination of each laboratory session, wipe bench tops with a disinfectant solution provided by the instructor.
4. Do not place contaminated instruments, such as inoculating loops, needles, and pipettes, on bench tops. Loops and needles should be sterilized by incineration, and pipettes should be disposed of in designated receptacles.
5. On completion of the laboratory session, place all cultures and materials in the disposal area as designated by the instructor.
6. Rapid and efficient manipulation of fungal cultures is required to prevent the dissemination of their reproductive spores in the laboratory environment.

To prevent accidental injury and infection of yourself and others, observe the following regulations at all times:

1. Wash your hands with liquid detergent and dry them with paper towels upon entering and prior to leaving the laboratory.
2. Wear a paper cap or tie back long hair to minimize its exposure to open flames.
3. Wear a lab coat or apron while working in the laboratory to protect clothing from contamination or accidental discoloration by staining solutions.
4. Closed shoes should be worn at all times in the laboratory setting.

5. Never apply cosmetics or insert contact lenses in the laboratory.
6. Do not smoke, eat, or drink in the laboratory. These activities are absolutely prohibited.
7. Carry cultures in a test-tube rack when moving around the laboratory. Likewise, keep cultures in a test-tube rack on the bench tops when not in use. This serves a dual purpose: to prevent accidents and to avoid contamination of yourself and the environment.
8. Never remove media, equipment, or especially, **bacterial cultures** from the laboratory. Doing so is absolutely prohibited.
9. Immediately cover spilled cultures or broken culture tubes with paper towels and then saturate them with disinfectant solution. After 15 minutes of reaction time, remove the towels and dispose of them in a manner indicated by the instructor.
10. Report accidental cuts or burns to the instructor immediately.
11. Never pipette by mouth any broth cultures or chemical reagents. Doing so is strictly prohibited. Pipetting is to be carried out with the aid of a mechanical pipetting device.
12. Do not lick labels. Use only self-stick labels for the identification of experimental cultures.
13. Speak quietly and avoid unnecessary movement around the laboratory to prevent distractions that may cause accidents.

The specific precautions outlined below must be observed when handling body fluids of unknown origin due to the possible imminent transmission of the HIV and hepatitis B viruses in these test specimens.

1. Disposable gloves must be worn during the manipulation of these test materials.
2. Immediate hand washing is required if contact with any of these fluids occurs and also upon removal of the gloves.
3. Masks, safety goggles, and laboratory coats should be worn if an aerosol might be formed or splattering of these fluids is likely to occur.
4. Spilled body fluids should be decontaminated with a 1:10 dilution of household bleach, covered with paper toweling, and allowed to react for 10 minutes before removal.
5. Test specimens and supplies in contact with these fluids must be placed into a container of disinfectant prior to autoclaving.

I have read the above laboratory safety rules and regulations and agree to abide by them.

Name _____ Date _____

Laboratory Protocol

STUDENT PREPARATION FOR LABORATORY SESSIONS

The efficient performance of laboratory exercises mandates that you attend each session fully prepared to execute the required procedures. Read the assigned experimental protocols to effectively plan and organize the related activities. This will allow you to maximize use of laboratory time.

PREPARATION OF EXPERIMENTAL MATERIALS

Microscope Slides: Meticulously clean slides are essential for microscopic work. Commercially precleaned slides should be used for each microscopic slide preparation. However, wipe these slides with dry lens paper to remove dust and finger marks prior to their use.

Labeling of Culture Vessels: Generally, microbiological experiments require the use of a number of different test organisms and a variety of culture media. To ensure the successful completion of experiments, organize all experimental cultures and sterile media at the start of each experiment. Label culture vessels with non-water-soluble glassware markers and/or self-stick labels prior to their inoculation. The labeling on each of the experimental vessels should include the name of the test organism, the name of the medium, the dilution of sample, if any, your name or initials, and the date. **Place labeling directly below the cap of the culture tube.** When labeling Petri dish cultures, only the name of the organism(s) should be written on the bottom of the plate, close to its periphery, to prevent obscuring observation of the results. The additional information for the indentification of the culture should be written on the cover of the Petri dish.

INOCULATION PROCEDURES

Aseptic techniques for the transfer or isolation of microorganisms, using the necessary transfer instruments, are described fully in the experiments in Part I of the manual. Technical skill will be acquired through repetitive practice.

Inoculating Loops and Needles: It is imperative that you incinerate the entire wire to ensure absolute sterilization. The shaft should also be briefly passed through the flame to remove any dust or possible contaminants. To avoid killing the cells and splattering the culture, cool the inoculating wire by tapping the inner surface of the culture tube or the Petri dish cover prior to obtaining the inoculum.

When performing an aseptic transfer of microorganisms, a minute amount of inoculum is required. If an agar culture is used, touch only a single area of growth with the inoculating wire to obtain the inoculum. **Never drag the loop or needle over the entire surface, and take care not to dig into the solid medium.** If a broth medium is used, first tap the bottom of the tube against the palm of your hand to suspend the

microorganisms. ***Caution:*** Do not tap the culture vigorously as this may cause spills or excessive foaming of the culture, which may denature the proteins in the medium.

Pipettes: Use only sterile, disposable pipettes or glass pipettes sterilized in a canister. The practice of **pipetting by mouth has been discontinued** to eliminate the possibility of auto-infection by the accidental imbibement of the culture or infectious body fluids. Instead, a mechanical pipetting device is to be used to obtain and deliver the material to be inoculated.

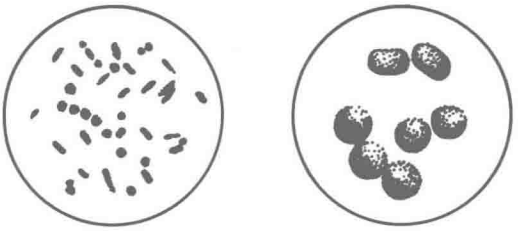
INCUBATION PROCEDURE

Microorganisms exhibit a wide temperature range for growth. However, for most used in this manual, optimum growth occurs at 37°C over a period of 18 to 24 hours. Unless otherwise indicated in specific exercises, incubate all cultures under the conditions cited above. Place culture tubes in a rack for incubation. Petri dishes may be stacked; however, they **must always be incubated in an inverted position (top down)** to prevent water of condensation from dropping onto the surface of the culture medium. This resultant excess moisture may then serve as a vehicle for the spread of the microorganisms on the surface of the culture medium, thereby producing confluent rather than discrete microbial growth.

PROCEDURE FOR RECORDING OBSERVATIONS AND RESULTS

The accurate accumulation of experimental data is essential for the critical interpretation of the observations upon which the final results will be based. To achieve this end, it is imperative that you complete all the preparatory readings that are necessary for your understanding of the basic principles underlying each experiment. Meticulously record all the observed data in the "Observations and Results" section of each experiment.

In the exercises that require drawings to illustrate microbial morphology, it will be advantageous to depict shapes, arrangements, and cellular structures enlarged to 5 to 10 times their actual microscopic size, as illustrated below. For this purpose a number 2 pencil is preferable. Stippling may be used to depict different aspects of cell structure, e.g., endospores or differences in staining density.



Poor drawing Good drawing

REVIEW QUESTIONS

The review questions are designed to evaluate understanding of the principles and the interpretations of observations in each experiment. Completion of these questions will also serve to reinforce many of the concepts that are discussed in the lectures. The designated critical-thinking questions are designed to stimulate further refinement of cognitive skills.

PROCEDURE FOR TERMINATION OF LABORATORY SESSIONS

1. All equipment, supplies, and chemical reagents are to be returned to their original locations.
2. All capped test-tube cultures and closed Petri dishes are to be neatly placed in a designated collection area in the laboratory for subsequent autoclaving.
3. Contaminated materials, such as swabs, disposable pipettes, and paper towels, are to be placed in a biohazard receptacle prior to autoclaving.
4. Hazardous biochemicals, such as potential carcinogens, are to be carefully placed into a sealed container and stored in a fume hood prior to their disposal according to the institutional policy.

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